RUBBER IN CRYPTOSTEGIA LEAF CHLORENCHYMA [1]

Robert T. Whittenberger and Albert Kelner [2]

ALTHOUGH RUBBER is a fairly common constituent of higher plants, most information concerning its origin, function, and anatomical occurrence has been obtained from investigation of *Hevea brasiliensis* Muell. Arg., or in more recent years, of *Parthenium argentatum* Gray and *Taraxacum kok-saghyz* Rodin (Lloyd, 1911, 1932; Bobilioff, 1923; Memmler, 1934; Spence and McCallum, 1935; Mazanko, 1938, 1940; Prokof'ev, 1939, 1940). Rubber in the dispersed or latex form occurs in laticiferous ducts in *Hevea* and *Taraxacum*, and in parenchyma and resin canal cells in *Parthenium*. The modes of rubber distribution in these plants have been accepted as prototypes. The possibility that rubber may occur also in regions other than those mentioned, that nonlatex as well as latex rubber may occur in the same plant, and that there may be some relation between rubber and such structures as chloroplasts has been overlooked.

With the wartime loss of *Hevea* rubber, other plants have been considered as sources of natural rubber. One of these, *Cryptostegia*, has abundant latex, and a well-developed system of laticiferous ducts ramifies throughout the entire plant (Dolley, 1911; Polhamus *et al.*, 1934; Viswanath *et al.*,

[1] Received for publication June 22, 1945.
Natural Rubber from Domestic Sources. Paper No. 9.
[2] Formerly of this Laboratory; now at the University of Pennsylvania, Philadelphia, Pa.
The chemical analyses, which required specially developed micro-techniques, were performed by C. O. Willits and C. L. Ogg. The assistance and cooperation of other members of this laboratory are also gratefully acknowledged.

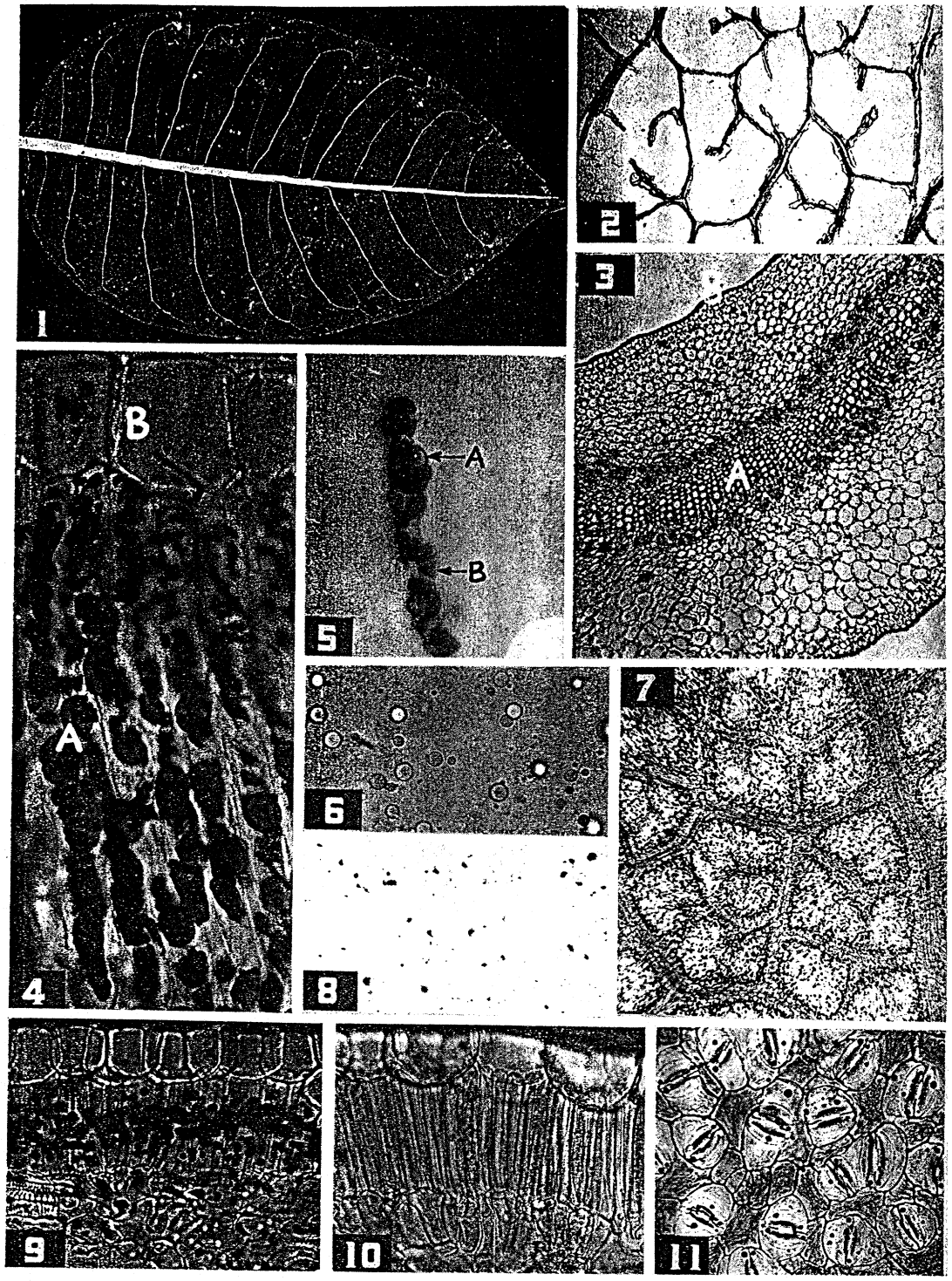

Fig. 1–11. Photomicrographs of *Cryptostegia* hybrid leaf.—Fig. 1. Vein pattern, ×1, obtained by retting the leaf and removing all tissues except the xylem.—Fig. 2. Xylem of small veins of retted leaf. ×50.—Fig. 3. Transverse section of midrib, acetone extracted. ×50. Stained areas above and below the xylem (A) represent rubber in laticiferous ducts. B, upper epidermis.—Fig. 4. Transverse section of fresh, living, mature leaf, mounted in water, untreated, unstained. ×460. Rubber-bearing globules (A) intermingled with the darker, smaller chloroplasts in the palisade cells are shown. B, upper epidermis.—Fig. 5. Unstained, naked, retted palisade protoplast showing several of the globules. ×940. A, single globule; B, chloroplast.—Fig. 6. Rubber-bearing globules isolated from the leaf chlorenchyma. ×460. Some globules are not in focus.—Fig. 7. Peridermal section of spongy mesophyll showing numerous

1943; Blaser, 1945). Heretofore it has been assumed that all the rubber of this plant occurred as latex in the duct system. However, during a study of the latex system of the leaves, it was noted that chlorenchyma cells contained large globules which stained intensely with oil-soluble stains such as Sudan III and oil blue NA (Whittenberger, 1944) but were like rubber in that they were insoluble in acetone and nonsaponifiable.

Because of the theoretical and practical importance of a possible relation between rubber and chlorophyll in this plant, an intensive study of these globules was initiated. Most investigations on the origin of rubber in plants having laticiferous ducts have centered exclusively upon the ducts as the site of rubber formation (Bobilioff, 1923; Mann, 1932; Mazanko, 1938; Blokhintseva, 1940; Prokof'ev, 1944; and others). Memmler (1934) stated that it had not yet been possible to detect rubber in other cells, especially in the parenchyma cells surrounding the laticiferous elements. Furthermore, previous workers on *Cryptostegia* (Dolley, 1911; Polhamus *et al.*, 1934; Viswanath *et al.*, 1943; Blaser, 1945) did not mention the presence of rubber in the leaf chlorenchyma.

MATERIAL AND METHODS.—The principal observations were made on leaves of *Cryptostegia* hybrid (*C. madagascariensis* Boj. × *C. grandiflora* R. Br.) and *Cryptostegia grandiflora* R. Br. Most of the plants were grown in Florida and shipped under refrigeration to Philadelphia at frequent intervals; other plants were sent from Cuba and Mexico. Supplemental observations were also made on fresh greenhouse plants.

Leaves were sectioned free hand or with a sliding microtome and examined either immediately in a drop of water, or after staining with Sudan III or oil blue NA. Although the globules could be seen in living, unstained leaf sections, they were most clearly brought out by the following procedure. Sections were treated with Javelle water for ten minutes, after which they were thoroughly washed in water, rinsed in 50 per cent ethanol, and immersed for 20 minutes in a saturated solution of oil blue NA in 50 per cent ethanol. They were then rinsed briefly in 50 per cent ethanol and in water and were examined either in water, glycerin, Karo, or glycerin jelly. Many observations, especially on the laticiferous ducts, were made with the dissecting microscope in order to avoid the displacement and loss of rubber attendant on the preparation of sections. Procedures for determining solubility and other characteristics are described later.

RESULTS.—*Distribution of latex ducts in the leaf.*—The laticiferous ducts are present in approximately equal numbers in the tissue above and below the leaf veins which they follow throughout the blade. Typical vein patterns are shown in figures 1 and 2, and a cross section of the midrib in figure 3. In a mature leaf, the ducts are most numerous (50 to 100) and largest (10 to 25 microns in diameter) along the midrib; they decrease gradually in number and size as the veins branch. Associated with each of the 20 to 25 largest lateral veins are 10 to 20 ducts of about 8 to 20 microns in diameter. Some ducts come in contact with the palisade parenchyma and epidermis (Blaser, 1945). Each of the smallest veins terminates with a single tracheid (fig. 2) and occasionally is devoid of ducts. Individual leaves differ widely in the number and size of ducts, and probably in the amount of latex rubber they contain.

Distribution of leaf globules.—The globules are clearly visible as colorless, hyaline, homogeneous masses even in unstained sections of living mature leaves (fig. 4). Figure 5 shows the globules in a retted palisade protoplast, and in figure 6 they are shown completely isolated. Frequently the larger globules are partly surrounded by chloroplasts. The globules occur in the cytoplasm of all the green mesophyll cells, although they are most numerous (1 to 8 per cell) in the palisade layer. In this layer they are uniformly distributed and of equal concentration throughout the leaf blade. In the dorsal half of the leaf (spongy parenchyma), the globule-bearing cells are more numerous adjoining the ducts than in nonduct areas (fig. 7).

The globules may attain a diameter of 10 to 12 microns (in the palisade cells) although smaller diameters (3 to 7 microns) are more common. They are therefore considerably larger than the rubber particles (fig. 8) of the leaf laticiferous duct system. It should be emphasized, however, that the globules vary greatly in size, frequency, and shape, depending upon the development, age, and previous history of the leaf. In young leaves there are only small globules, or none at all; in mature leaves of unusually high rubber content, the chlorenchyma cells are heavily laden with globular material. In the latter cells, many of the globules are distended to form oval, sheet-like, or rod-shaped masses. A rapid estimate of the quantity of rubber in a leaf may be made by noting microscopically the extent of the globular material of the cell.

Character of the unextracted leaf globules.—There are no accurate, direct, microchemical tests for determining rubber in small plant cells. Oil blue NA and other oil-soluble stains do not differentiate rubber from lipids, cutin, resins, suberin, and various waxes, and solubility tests alone are inconclusive. For preliminary determinations, however, the

rubber-bearing globules and channels of lower epidermis exposed by the removal of the veins. Bleached with Javelle water, stained with oil blue NA. ×50.—Fig. 8. Electron micrograph of latex from the leaf laticiferous duct system. ×1280.—Fig. 9. Transverse section, bleached with Javelle water, acetone-extracted for 48 hours, and stained with oil blue NA. ×215. Stained bodies in the chlorenchyma are rubber.—Fig. 10. Transverse section, bleached with Javelle water, ether-extracted for 24 hours, and stained with oil blue NA. ×200. No rubber remained after ether extraction.—Fig. 11. Lower epidermis showing globules in the guard cells. Bleached with Javelle water, stained with oil blue NA. ×215.

collective use of staining and solubility tests consistently indicated that the globules contained rubber.

Stains such as oil blue NA, Sudan III, Sudan IV, oil red N-1700, or alkanet applied directly to the leaf stained these globules intensely, sharply differentiating the larger ones from the chloroplasts, although some of the smaller globules were obscured. The globules could be more distinctly demonstrated if the cells were first cleared and bleached with Javelle water or sodium hypochlorite solution. Upon treatment with Javelle water, the chloroplasts and even the protoplasts disintegrated and disappeared, whereas the large globules remained essentially unchanged. As certain chloroplasts disintegrated, small bright globules, which previously could not be observed, appeared in their places. These were apparently identical in nature with the original globules in the intact cell, and took the rubber stains in the same manner. It was uncertain whether these new globules were originally distinct but hidden by the chloroplasts, or whether they were formed from the disintegrating chloroplastic material.

Solubility of globules was determined by putting sections into absolute ethanol or acetone and then into particular solvents, in which they were kept at room temperature for 24 to 48 hours, or at boiling temperature for 8 to 16 hours. They were then again put into acetone or ethanol, washed with water, treated with Javelle water, washed with water again and then with 50 per cent ethanol, stained, and examined. Usually, however, it was more convenient to treat sections with Javelle water first and, after extraction, to examine the sections directly in acetone or ethanol without staining. Control experiments showed that treatment with Javelle water did not alter the solubility of the globules. Solvents of relatively high polarity were applied directly to untreated sections and were permitted to act from two to four hours.

Table 1 shows that the globules had the characteristics of rubber. That they were not composed largely of resins was indicated by their insolubility in ethanol, acetone, sulfuric acid, and chloral hydrate; that they were not waxes or fat globules was suggested by their insolubility in acetone (fig. 9) and their nonsaponifiability. The globules were distinguished from gums and mucilages by being insoluble in boiling water, chloral hydrate, and Javelle water, and soluble in ether (fig. 10). Moreover, they dissolved in the common rubber solvents, such as benzene, xylene, and carbon tetrachloride.

The behavior of the globules in acetone and ether was characteristic enough to merit particular discussion. Acetone caused some of the globules to lose their characteristic globular morphology, shrink somewhat, and stick in patches to the cell wall (fig. 9). These bodies stained as brilliantly as ever with oil blue NA. Drastic acetone extraction carried out for 32 hours at 25°C. and for 16 hours at boiling temperature in no way altered this picture.

TABLE 1. *Effect of various treatments on globules in Cryptostegia hybrid leaf.*

Treatment	Observable effect on globules
Distilled water	None
95% ethyl alcohol	None
Absolute alcohol	None
Methyl alcohol	None
Acetone	Shrink moderately, some stick to cell wall (see text)
Ethyl ether	Dissolve (see text)
Benzene	Dissolve (see text)
Xylene	Dissolve
Carbon tetrachloride	Dissolve
Carbon disulfide	Dissolve
Chloroform	Dissolve imperfectly
Glacial acetic acid	Appear finely pitted
10% potassium hydroxide in 95% ethanol	Shrink slightly, but are not saponified
Potassium hydroxide-ammonia reagent	Shrink slightly, but are not saponified
Aqueous chloral hydrate	None
5% sulfuric acid	None
72% sulfuric acid	Swell slightly, but remain homogeneous
Javelle water	Appear finely pitted
10% ferric chloride	Negative, tannin absent

Since Spence and Caldwell (1933) have shown that sometimes fats are rendered acetone-insoluble by admixture with proteins, sections were treated in the following manner to hydrolyze and remove proteins before acetone extraction:

(1) Water extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
(2) 5 per cent H_2SO_4 extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
(3) Water extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
(4) Acetone extraction for 32 hrs. at 25°C., + 16 hrs. boiling.

After step 4, staining of sections revealed that globules with characteristic "acetone" morphology were still present. Finally, after step 4, sections were extracted with benzene for 32 hours at 25°C., plus 16 hours at the boiling temperature. No globules were present in the somewhat macerated sections.

When sections were extracted in ether or benzene for short periods, 18 hours or less at 25°C., another characteristic picture developed. In one experiment, for example, sections were treated with Javelle water, then ether-extracted for 18 hours at 25°C. The cells appeared entirely empty when examined in ether (fig. 10). These sections were then transferred directly to a drop of absolute acetone on the slide and examined. The cells became filled with myriad tiny particles in active Brownian motion, which stained brilliantly with oil blue NA. In another experiment in which sections were given the same treatment except that they were ether-extracted for 48 hours, the addition of acetone

caused no formation of colloidal particles. It was probable that ether treatment for 18 hours at 25°C. dissolved the globules, perhaps filling the cell with a viscous solution which did not penetrate the cell wall readily. Subsequent addition of acetone precipitated the globules in colloidal form within the cell. These colloidal particles redissolved when ether was added, but were reprecipitated by acetone. Since the refractive index of acetone, 1.35, is almost identical with that of ether, 1.36, it is apparent that the phenomenon cannot be explained by a difference in refractive index of the two solvents.

A similar experiment carried out with benzene instead of ether was less satisfactory because the entire section was quite transparent. However, a fine suspension appeared within the cells when the sections were treated with acetone. It should be emphasized that this phenomenon appeared only in fairly thick sections (50 to 100 μ) extracted for 18 hours or less in the solvent at room temperature. Longer extraction, or heat, rid the cell of all ether- or benzene-soluble material, and no precipitation by acetone occurred.

Further information was obtained by micrurgical tests. Globules were obtained free of cell walls by mechanical disintegration of leaves softened with dilute alkali. When a single globule was subjected to tension by two micro-needles, it stretched about tenfold before rupture. After rupture, the stretched threads quickly retracted, and two separate globules were formed. The globule, however, was tacky and when punctured stuck to the glass needle, suggesting the presence of rubber of low molecular weight. These staining, solubility, and micrurgical tests strongly indicated, although they did not prove, that the chlorenchyma globules contained rubber.

Chemical analysis of dissected leaf fractions.— Chemical analysis of an entire *Cryptostegia* hybrid leaf showed that it contained about 3 to 5 per cent of rubber on the dry-weight basis. It was difficult to determine what portion of this rubber was due to the latex in the leaf, and what portion, if any, was contributed by the globules in the chlorenchyma. Evidence on this point could be obtained by separate chemical analysis of the latex-bearing and nonlatex-bearing portion of the leaf. However, obtaining chlorenchyma cells free of latex ducts was a task soon abandoned because of the difficulty of isolating them in quantity sufficiently large for chemical analysis. Rather, the following experiment was carried out.

From each of six *Cryptostegia* hybrid twigs three leaves were plucked, and the sample was divided into three lots, each of which contained only one leaf from any given twig. Only uninjured, nonchlorotic, turgid, clean leaves were selected. The leaves were severed at the abscission layer, and no latex was observed to have been lost through exudation. They were infiltrated under suction successively with dilute acetic acid, water, and dilute sodium hydroxide in order to coagulate any dispersed latex and to soften them sufficiently for dissection.

Since previous anatomical studies had indicated that the quantity of latex duct rubber was closely correlated with the quantity of veins, it was believed that removal of the main vein would remove a like proportion of the latex ducts. In a separate experiment on retted leaves (see fig. 1 and 2 for vein pattern), it was shown that the main vein and petiole contained by weight more than half the xylem of all the veins of the leaf, and that the xylem of the 20 to 25 largest lateral veins contributed only a small fraction of the total weight. Accordingly, two of the three lots of leaves were dissected with micro-scalpels under the stereoscopic microscope. The first lot was separated into two fractions; 1a comprised the petiole and midrib with its associated ducts, and 1b comprised the remainder of the leaf tissues. The second lot was divided into three fractions: 2a was identical with 1a; 2b comprised the 24 largest lateral veins and associated ducts of each leaf; and 2c comprised the remainder of the leaf tissues, including the smallest veins and ducts. The third lot was not dissected and served as a control. Since the globule-bearing cells are most numerous near the veins and ducts (fig. 7), extreme care was used in all cases in separating these cells from the vein-duct fraction. All fractions were analyzed for total rubber, with the results shown in table 2.

The unusually high figures for the benzene extract were undoubtedly due to the removal from the leaves of some of the nonrubber plant constituents by the acid, water, and alkali treatment before dissection. However, as shown in the last column, the

TABLE 2. *Analysis of dissected Cryptostegia hybrid leaf fraction.*

Leaf fraction	Weight of leaf fraction, g.[a]	Resin (acetone extract), per cent[a]	Rubber (benzene extract), per cent[a]	Per cent of total leaf rubber
1(a) Petiole and midrib	0.177	7.5	6.6	9.0
1(b) Remaining leaf tissue	1,248	14.7	9.5	91.0
2(a) Petiole and midrib	0.157	8.9	4.3	5.3
2(b) Largest lateral veins	0.038	13.9	5.0	1.5
2(c) Remaining leaf tissue	0.934	15.8	12.5	93.2
3 Undissected leaf	1.061	15.4	9.7	100

[a] Moisture-free basis.

distribution of rubber was not affected by this pretreatment. It is concluded that 85 to 90 per cent of the total rubber of these leaves occurred in chlorenchyma cells, that only 10 to 15 per cent existed in the latex ducts, that the ducts of the largest lateral veins contained only an insignificant fraction of the total rubber, and that most of the latex rubber in the leaves occurred in the petiole and midrib. Furthermore, on the basis of the data above and the chemical analysis of plant organs for rubber (Viswanath *et al.*, 1943), it appears that the leaf chlorenchyma globules contain the greater portion of the total rubber in the average three or four year old plant, the smaller portion occurring as latex in the stem, leaf, and root.

To estimate that 85 to 90 per cent of the total leaf rubber occurred in chlorenchyma cells, it was necessary to assume that latex rubber was no more concentrated in the small ducts in the undissected portions of the blade than in the dissected duct fractions. Microscopic examination indicated that the latex rubber was actually more concentrated in the larger ducts associated with the larger veins. In fact, some of the smallest ducts of the smallest veins appeared to contain structureless material similar to that of the globules rather than typical latex. It has already been pointed out that on the average the ducts are largest and most numerous along the largest veins. It seemed justified, therefore, on the basis of the total amount of leaf rubber in the ducts of the petiole and midrib (average 7.2 per cent) and in the 24 largest lateral veins (1.5 per cent), to assume that even less than 7.2 per cent of the total rubber remained in the small, unseparated ducts of the blade. The estimate that 85 to 90 per cent of the total rubber occurred in the leaf chlorenchyma receives independent support from data obtained during research on the recovery of rubber from *Cryptostegia* (Whittenberger, Brice and Copley, 1945).

The microscopic observation that individual leaves vary widely in the amount of latex rubber they contain is substantiated by the data in table 2. For example, the latex ducts associated with the main vein and petiole of lot 1 contained 9.0 per cent of the total leaf rubber, whereas those of lot 2 contained only 5.3 per cent of the total. The main vein and petiole fraction of lot 1 also contained rubber in greater concentration (6.6 per cent) and resin in less concentration (7.5 per cent) than did the corresponding fraction of lot 2 (4.3 per cent rubber and 8.9 per cent resin). These facts suggested that the average age of lot 1 was greater than that of lot 2, since the ratio of rubber to resin and oil becomes greater as the plant grows older (Prokof'ev, 1939; Blokhintseva, 1940; Moshkima, 1940).

Properties and composition of isolated globules. —Although data indicated that the bulk of the rubber occurred in leaf cells exterior to the laticiferous duct system, they were not entirely conclusive. The possibility existed that the chemical method of analysis (based upon the fact that rubber is soluble in benzene and insoluble in acetone) was incapable of distinguishing rubber from another substance or combination of substances which possessed certain properties similar to those of rubber. Moreover, since the leaf was not completely dissected, exactly how much latex rubber was mixed with the cell rubber was not determined. Conclusive proof that the leaf chlorenchyma globules contained rubber could be obtained by isolating the globules in quantity sufficient for physical tests and x-ray analysis. With the development of a method for the recovery of rubber from *Cryptostegia* leaves by Naghski and associates (1945), a means of isolation of the globules became available. During the isolation special precautions were taken to assure that the separation of the cell globules from the latex rubber was complete. The effect of each step of the process on the globules and latex was followed microscopically. The isolated mass of chlorenchyma globules was yellowish, soft, tacky, and elastic, and exhibited noticeable snap when stretched and released. The results of chemical analysis of the globules are shown in table 3.

TABLE 3. *Chemical analysis of fresh mature Cryptostegia hybrid leaves (starting material) and the globules isolated from the leaf cells.*

	Original leaves	Isolated cell globules
Wet weight, grams	3,350	11.4
Dry weight, grams	450	8.7
Moisture, per cent	86.5	24.3
Acetone solubles by direct extraction, per cent[a]	9.4	29.6
Rubber hydrocarbon in acetone solubles, per cent[a]	.....	3.8
Acetone solubles, nonrubber, per cent[a]	.....	25.8
Acetone and benzene insolubles, per cent[a]	.....	1.3
Total rubber hydrocarbon, per cent[a]	3.5	65.0
Nonrubber benzene-soluble acetone-insolubles, by difference, per cent[a]	.....	7.9

[a] Moisture-free basis.

About 65 per cent of the globular material was rubber. About 8 per cent of the isolated globules was nonrubber, benzene-soluble, acetone-insoluble material of unknown composition. Rubber hydrocarbon in the acetone extract, as indicated by the analysis, might be expected if rubber of low molecular weight is present in the globules. It was determined gravimetrically by precipitating the hydrocarbon as rubber "tetrabromide" and establishing the identity of the precipitate by bromide analysis. Hoover *et al.* (1945) found that 93 per cent of the globular material was soluble in methyl ethyl ketone, an indication of the low degree of polymerization of the rubber (Cheyney, 1942). Spectrophotometric examination of the benzene solution of the globules indicated the presence of pheophytin and carotene.

Another portion of the globular material, after being compounded by a modified A.C.S. formula containing 50 parts of channel black, was vulcanized and tested for rubberlike properties. The properties of the vulcanizate—ultimate elongation, modulus at 300 per cent elongation, and tensile strength —were characteristic of rubber of low molecular weight. Later and more detailed information on the character of this rubber has been published by Hoover *et al.* (1945).

Final proof of the existence of rubber in the isolated cell globules was furnished by x-ray diffraction studies of the vulcanizate. At 320 per cent elongation, slight arcing of the innermost amorphous ring became evident. At 450 per cent elongation, eight crystalline reflections became visible. The interplanar spacings determined from these discrete reflections agreed with those of stretched *Hevea* rubber, indicating the presence of a *cis*-polyisoprene molecular structure.

Discussion.—The chlorenchyma rubber in *Cryptostegia* leaf represents a hitherto unrecognized mode of rubber distribution and storage in this plant. Whether leaf chlorenchyma rubber is as widely distributed among plants as is latex rubber, or whether other plants exist in which both laticiferous duct and leaf chlorenchyma rubber occur simultaneously, is not known. Naylor (1943) found rubber both in ducts (long rod-like cells) and in the mesophyll parenchyma of *Eucommia ulmoides* Oliver. Little detail is given, however, as to the method by which he determined that the globular masses in the parenchyma were rubber. Novikov *et al.* (1934) stated that in *Scorzonera tau-saghyz* Lips. & Bos. and in *Apocynum venetum* L. rubber may be detected in the green parenchyma of the leaf notwithstanding the latex cells in the leaf. Kiselev *et al.* (1934) also reported that rubber is formed in the photosynthetic tissue of *Scorzonera tau-saghyz* Lips. & Bos. and *Chondrilla* spp., and passes from there in an unknown form into the latex ducts. These observations, however, have not been generally accepted and are at variance with the findings of Prokof'ev (1939, 1944).

Although Lloyd (1911) reported finding rubberlike bodies in the leaf chlorenchyma of *Parthenium argentatum* Gray, subsequent workers (Artschwager, 1943; Moshkima, 1940) do not mention similar bodies in the leaves of this plant. The rubber occurring in the stem chlorenchyma of *Parthenium* (Spence, 1928, 1938; Lloyd, 1932) is a latex markedly different from the nonlatex rubber reported here, and probably bears a different relationship to chlorophyll. In *Chrysothamnus* spp. Hall and Goodspeed (1919) reported that one or more rubber globules may be detected in each of the palisade cells. There are no laticiferous ducts in this plant. Little detailed information is available concerning the anatomical disposition of rubber in *Solidago* spp. other than that it occurs principally in the leaves (Polhamus, 1933) and apparently is localized in parenchymatous tissues and in certain isolated areas (Legros, 1937). Our preliminary studies of *Solidago* spp. showed acetone-insoluble, rubberlike globules in the palisade and spongy mesophyll cells. These globules appeared similar to those in *Cryptostegia* leaf. However, no laticiferous duct rubber was observed.[3]

Our anatomical studies on the leaves of *Taraxacum kok-saghyz* Rodin revealed oil or resinlike globules in the chlorenchyma. These globules, accordingly, differed from those in *Cryptostegia* leaf in that they contained no rubber and were soluble in ethanol and acetone. All the leaf rubber was found in the laticiferous ducts. The possibility, however, that at least some of the latex rubber is synthesized in the leaf ducts is not denied by these observations. Therefore, they do not necessarily support the conclusion of Blokhintseva (1940) that the rubber of this plant is synthesized in the latex vessels of the root and that the synthesis bears no direct connection with the assimilating organs.

For obvious reasons, demonstration of leaf chlorenchyma rubber, especially in the presence of latex rubber as in *Cryptostegia*, was less simple than the demonstration of latex rubber alone. While all available staining and microchemical tests indicated that the globules contained rubber, final proof was obtained by x-ray analysis, which showed the *cis*-polyisoprene molecular structure. Confirmatory evidence was obtained during experimentation on methods for the large-scale recovery of rubber from the leaves (Naghski *et al.*, 1945; Whittenberger *et al.*, 1945).

The association of rubber with chloroplasts raises the question of the relationship between the food-manufacturing organs and chlorenchyma rubber. Since young leaves lack globules, and globules form and enlarge as the leaves mature, it is probable that the rubber is accumulated slowly within the cells and requires an excess of photosynthetic products. Thus the palisade cells with their abundance of chloroplasts probably accumulate a greater excess of photosynthetic products, and hence rubber, than the spongy mesophyll cells. The apparent increase in the number of rubber-bearing globules brought about by the disintegration of chloroplasts by Javelle water suggests the possibility that some rubber is formed within the chloroplast itself, in a manner analogous to starch formation within chloroplasts or oil formation within eliaoplasts. Prokof'ev (1939) and Blokhintseva (1940) are of the opinion that the synthesis of latex rubber in *Taraxacum kok-saghyz* Rodin probably occurs in the plastids of the laticiferous ducts.

There can be little doubt that the chlorenchyma rubber originates in the leaf. This rubber appears in no other part of the plant. There is a question, however, concerning the origin of the widely dis-

[3] Recently M. E. Rollins, T. L. W. Bailey, Jr., and I. V. deGruy (unpublished report at the Southern Regional Research Laboratory, New Orleans, La., 1945) have shown that the rubber in *Solidago leavenworthii* Torr. & Gray occurs as small globules, 1 to 5 per cell, in the spongy mesophyll and palisade cells of the leaf.

tributed latex rubber. Viswanath *et al.* (1943), on the basis of studies on the quantity and composition of latex before and after the defoliation period, suggest that the latex rubber of *Cryptostegia grandiflora* R. Br. originates in the organs of photosynthesis. If this suggestion is accepted, then it is permissible, on the basis of our present knowledge of the character and disposition of the chlorenchyma globules, to speculate on a possible relation between the globules and the duct rubber. The investigators named above evidently were unaware of the chlorenchyma globules. It is conceivable that the globule rubber is deposited in chlorenchyma cells by a mechanism analogous to that which causes the accumulation of starch in leaf chlorenchyma of many plants. This relatively unstable rubber might then be digested to such a state that its translocation to the ducts would be possible. Within the ducts synthesis of large rubber molecules could occur, resulting in the formation of latex rubber of good quality. Translocation and synthesis could occur at a substantially continuous and rapid rate, constantly replenishing the latex rubber as it is withdrawn from the plant every one to three days (Symontowne, 1943; Fennell, 1944). Such a conception would account for the observed diminution in latex flow upon defoliation, and is consistent with the observation that the degree of polymerization of rubber of the leaf ducts apparently is higher than that of the chlorenchyma cells (Hoover *et al.*, 1945). Micrurgical evidence indicates that the degree of polymerization of the leaf duct rubber is intermediate between that of the stem ducts and chlorenchyma cells.

In this conception, an early precursor of latex rubber, that is, the material translocated from the cells to the ducts, would presumably consist of a compound less oxidized than simple sugars.[4] Mann (1932) stated that no simple sugars have been isolated from the latex of *Hevea brasiliensis* Muell. Arg., although he held the opinion that the latex rubber was synthesized directly from temporary simple sugars in the ducts.

While *Cryptostegia* may be outstanding in possessing leaves that contain both laticiferous duct and chlorenchyma rubber, it is not unique in having the major portion of its leaf rubber low in molecular weight. There is evidence also that the leaf rubber of *Asclepias syrica* L. (Paul, Blakers, and Watson, 1943) and *Solidago leavenworthii* Torr. & Gray (Skau *et al.*, 1945) is of low molecular weight. Such a condition might be expected if it is admitted that a large portion of material synthesized in the leaf, whether rubber or otherwise, is rapidly translocated, in simple form and by some as yet unknown mechanism, to the more permanent parts of the plant.

[4] There is evidence that the precursor complex may include lupeol esterified with two hydroxy-n-fatty acids (unpublished report by S. G. Wildman, S. B. Hendricks, F. A. Abegg, J. A. Elder, and P. E. Heath of the Bur. Plant Ind., Soils and Agric. Eng. at Beltsville, Maryland, 1945).

In view of the close relationship between *Cryptostegia* chlorenchyma rubber and photosynthetic activity of the cell it is difficult to see how this rubber can be merely a waste product or a wound-healing agent, or serve in many of the other roles commonly suggested for latex rubber. It is far more likely that this rubber is a storage product of definite physiological significance. Whether it represents a highly efficient form of food storage, whether its importance lies in its ability to remove osmotically active simple sugars from the actively photosynthesizing cell, whether it is the precursor of latex rubber, and what factors determine its degree of polymerization are questions which must await physiological experimentation.

An interesting corollary of the observation that rubber and chlorophyll co-exist in the same cell in *Cryptostegia* leaf mesophyll is the absence of similar globules in the guard cells of the lower epidermis. Although globules are clearly visible in the guard cells of both fresh and Javelle water-treated strips of the epidermis (fig. 11), tests on these globules indicate that they are different from those of the mesophyll chlorenchyma, as only a few, if any, of them contain rubber. For the most part they are dissolved by ethanol or acetone and are removed by alcoholic potassium hydroxide. These observations, therefore, indirectly support the conclusion of Sayre (1926) that the green pigment of the guard cells is not identical with the chlorophyll of the leaf mesophyll. Otherwise, it might be expected that rubber globules similar to those in the mesophyll would occur also in the guard cells, since the globules in the mesophyll are correlated with the presence of chlorophyll.

SUMMARY

An unusual type of rubber storage has been found in *Cryptostegia* leaf. Although *Cryptostegia* possesses abundant rubber latex and a laticiferous duct system well developed in all parts of the plant, the major portion of the leaf rubber occurs as nonlatex globules in the mesophyll chlorenchyma, entirely distinct from the duct system. These globules are strikingly correlated with the presence of chlorophyll, being largest (up to 12 μ in diameter) and most numerous (as many as 8 per cell) in the palisade cells of fully mature leaves. The physiological significance of the association of the globules with chlorophyll is not yet known.

In mature *Cryptostegia* hybrid leaves, 85 to 90 per cent of the total rubber is in the chlorenchyma, the remaining 10 to 15 per cent occurring as latex in the laticiferous ducts. The ducts follow the veins throughout the blade and are largest and most numerous along the midrib.

Proof that the leaf chlorenchyma globules contained rubber was established by x-ray studies of the isolated globules, after exhaustive staining and microchemical tests on leaf sections. The chlorenchyma rubber, although possessing a *cis*-polyisoprene molecular structure, apparently is of lower

molecular weight than that of the laticiferous ducts. The globules contain about 65 per cent of rubber hydrocarbon, the remaining 35 per cent consisting largely of acetone-soluble material (resins).

EASTERN REGIONAL RESEARCH LABORATORY,
BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
PHILADELPHIA 18, PENNSYLVANIA

LITERATURE CITED

ARTSCHWAGER, ERNST. 1943. Contribution to the morphology and anatomy of guayule (*Parthenium argentatum*). U.S.D.A. Tech. Bull. No. 842, 1–33.

BLASER, H. W. 1945. Anatomy of *Cryptostegia grandiflora* with special reference to the latex system. Amer. Jour. Bot. 32: 135–141.

BLOKHINTSEVA, I. I. 1940. Formation of rubber in koksaghyz as a result of the functioning of the latex vessels. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 4, 608–613.

BOBILIOFF, W. 1923. Onderzockingen over het voorkomen van caoutchouc en melksapvaten in de bladeren van *Hevea brasiliensis*. Arch. Rubbercultuur 7: 205–215.

CHEYNEY, L. E. 1942. Methyl ethyl ketone extraction of rubber. Ind. Eng. Chem. 34: 1426–1428.

DOLLEY, C. S. 1911. On the occurrence of palay rubber (*Cryptostegia grandiflora* R. Br.) in Mexico. India Rubber Jour. 41: 1156–1158.

FENNELL, T. A. 1944. *Cryptostegia* rubber—its rebirth and development. Rubber Age 54: 329–332.

HALL, H. M., AND T. H. GOODSPEED. 1919. A rubber plant survey of western North America. Univ. California Publ. Bot. 7: 239–243.

HOOVER, S. R., T. J. DIETZ, J. NAGHSKI, AND J. W. WHITE, JR. 1945. *Cryptostegia* leaf rubber. Ind. Eng. Chem. 37: 803–809.

KISELEV, N. N., A. P. OSIPOV, AND K. A. KUZMINA. 1934. Conditions for the formation of rubber and resin and their movements in plants. Bull. Acad. Sci. U.R.S.S., classe Sci. Math. Nat. No. 9, 1367–1385.

LEGROS, J. 1937. Secondary rubber yielding plants of the Caucasus region and of Central Asia. Internat. Rev. Agr. 28: 468–481T.

LLOYD, F. E. 1911. Guayule (*Parthenium argentatum* Gray), a rubber plant of the Chihuahuan desert. Carnegie Inst. Washington Publ. No. 139: 1–213.

———. 1932. Mode of occurrence of caoutchouc in the guayule *Parthenium argentatum* Gray, and its function. Plant Physiol. 7: 131–138.

MANN, C. E. T. 1932. Latex production in relation to plant physiology. India Rubber Jour. 84: 653–656.

MAZANKO, F. P. 1938. Rubber formation in tau-saghyz. Compt. Rend. Acad. Sci. U.R.S.S. 19: 99–102.

———. 1940. Consumption of their rubber by certain plants. Compt. Rend. Acad. Sci. U.R.S.S. 27: 838–840.

MEMMLER, K. 1934. (Editor.) The Science of Rubber. Authorized English translation by R. F. Dunbrook and V. N. Morris. Reinhold Publishing Corp., New York.

MOSHKIMA, M. S. 1940. Structural peculiarities of the guayule rubber plant. Bull. Acad. Sci. U.R.S.S., Ser. Biol., No. 4, 614–620.

NAGHSKI, J., J. W. WHITE, JR., S. R. HOOVER, AND J. J. WILLAMAN. 1945. Anaerobic fermentation of *Cryptostegia* leaves for rubber recovery. Jour. Bact. 49: 563–574.

NAYLOR, E. E. 1943. Rubber from a hardy tree. N. Y. Bot. Gar. Jour. 44: 11–13.

NOVIKOV, V. A., A. GRECHUSHNIKOV, AND J. BARMENKOV. 1934. Accumulation of rubber in the roots of tau-saghyz as a result of its disappearance from the leaves. Compt. Rend. Acad. Sci. U.R.S.S. [N.S.] 1: 205–207.

PAUL, E. B., A. L. BLAKERS, AND R. W. WATSON. 1943. The rubber hydrocarbon of *Asclepias syrica* L. Can. Jour. Research 21: 219–223.

POLHAMUS, L. G. 1933. Rubber content of various species of goldenrod. Jour. Agric. Research 47: 149–152.

———, H. H. HILL, AND J. A. ELDER. 1934. Rubber content of two species of *Cryptostegia* and of an interspecific hybrid in Florida. U.S.D.A. Tech. Bull. No. 457.

PROKOF'EV, A. A. 1939. Rubber formation in plants. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 6, 908–923.

———. 1940. Biological role of rubber. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 4, 589–607.

———. 1944. On the possibility of rubber formation by plants in heterotrophic nutrition with carbohydrates. Compt. Rend. Acad. Sci. U.R.S.S. 43: 170–173.

SAYRE, J. D. 1926. Physiology of the stomata of *Rumex patientia*. Ohio Jour. Sci. 26: 233–267.

SKAU, E. L., W. J. RUNCKEL, F. B. KREEGER, AND M. A. SULLIVAN. 1945. Physical chemical investigations of goldenrod rubber. III. The fractionation of goldenrod and other natural rubbers. Jour. Phys. Chem. 49: 304–315.

SPENCE, D. 1928. Recent scientific advances in connection with guayule. Rubber Age 23: 133–134.

———. 1938. Preparation of latex from rubber-producing plants. U. S. Patent 2,119,030.

———, AND M. L. CALDWELL. 1933. Determination of rubber in rubber-bearing plants. Ind. Eng. Chem., Anal. Ed. 5: 371–375.

———, AND W. J. MCCALLUM. 1935. The function of the rubber hydrocarbon in the living plant. Trans. Inst. Rubber Ind. 11: 119–134.

SYMONTOWNE, R. 1943. *Cryptostegia* research in Haiti. India Rubber World 108: 148–150, 259–261.

VISWANATH, B., *et al.* 1943. *Cryptostegia grandiflora* R. Br. A wartime source of vegetable rubber. Jour. Sci. Ind. Research (India) 1: 335–383.

WHITTENBERGER, R. T. 1944. Oil blue NA as a stain for rubber in sectioned or ground plant tissues. Stain Tech. 19: 93–98.

———, B. A. BRICE, AND M. J. COPLEY. 1945. Distribution of rubber in *Cryptostegia* as a factor in its recovery. India Rubber World 112: 319–323.